

The University of Chicago

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FUNCTIONS OF THE EPIDERMAL
AND DERMAL COMPONENTS OF
THE PAPILLA IN FEATHER
REGENERATION

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

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PHYSIOLOGICAL ZoöLOGY
Vol. XVI, No. 4, October, 1943

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Volume XVI

OCTOBER 1943

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THE MORPHOGENETIC FUNCTIONS OF THE EPIDERMAL AND DERMAL COMPONENTS OF THE PAPILLA IN FEATHER REGENERATION¹

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I. INTRODUCTION

THE present study is an outgrowth of earlier experiments (Lillie and Wang, 1941) on the analysis of feather morphogenesis. The problem arose from the fact that the papilla is of composite origin, consisting of a dermal core and a thin covering of follicular epidermis, the so-called "regeneration cells"² (Lillie and Juhn, 1938). Since the feather proper is formed exclusively by the epi-

dermal cells, the question arises whether the dermal core of the papilla participates in morphogenesis in addition to its well-known nutritive function (Lillie, 1940). Lillie and Wang (1941) ascribed a fundamental morphogenetic function to the dermal component of the papilla, as a result of their experiments, viz., the determination of the symmetry and orientation of the feather by means of induction of bilateral organization in the ectoderm of the papilla, which was regarded as originally totipotent. But, as both derma and epidermis were involved in the experiments, the proof of this conclusion remained indirect.

Although it was found that breast and saddle papillae of the Brown Leghorn male or capon, when transplanted reciprocally between breast and saddle tracts, give rise to feathers specific to their respective donor tracts in both structure and pigmentation, this did not reveal whether the persistence of the donor tract specificity in the graft was due to its dermal or its epidermal constituent. These experiments, as well as others (feather germs transplanted under

¹ This research was done under the direction of Dr. Paul Weiss in partial fulfilment of the requirements for the degree of Doctor of Philosophy. It has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. Preliminary reports appeared in the *Anatomical Record* (Wang, 1941, 1942).

² The term "regeneration cells" is used throughout this paper as equivalent to the epidermal component of the papilla. These cells give rise to the entire epidermal coat of the papilla from which the collar of a regenerating feather is derived; however, owing to the fact that they represent a thickened portion of the follicular epidermis adjacent to the papilla, their existence does not terminate sharply at the boundary of the papilla but continues into the epidermis of the bottom of the follicle, i.e., surrounding the base of the papilla (see Lillie and Wang, 1941, Fig. 3). The latter portion of regeneration cells remains with the follicle when the papilla is removed.

the skin and chimaera feathers from combined sagittal half-papillae of different tracts), prove that an intact papilla or even part of it, but with both the epidermal and dermal components, regenerates true to its tract of origin, no matter where it may have been transplanted autoplastically. It remains to be decided, however, whether tract specificity is determined by either the dermal or the epidermal component to the exclusion of the other, or possibly by both jointly. The fact that the epidermal cells actually build the complete feather does not necessarily exclude the possibility of induction of tract specific characters by the dermal component. The question cannot be settled so long as the transplanted papillae are tested with both the epidermal and the dermal components coming from the same tract.

In order to delimit the morphogenetic roles of the two components of the papilla, a technique was devised by which the components of breast and saddle papillae could be separated and recombined. This was achieved by destroying the epidermal coat of a papilla and transplanting the denuded dermal core autoplastically into a follicle of the reciprocal tract, the papilla of which had been previously completely removed. Local epidermis of the host tract grew over the transplant, and a composite papilla was thus obtained, with the dermal portion coming from one tract and the epidermal portion from the other. The resulting feather was studied to ascertain the extent to which the contributing tracts had determined its characteristics.

Criteria used for differentiating breast and saddle feathers in Brown Leghorn capons are: shape, form, total length, proportion of fluff, and the structure of the barbs. In addition, growth rate and pigmentation, which are specific for the two tracts, were also used as diagnostic

features. The use of pigmentation in this connection was limited, however, by the lack of definite knowledge of the site of the potential melanophores in the definitive papilla (Willier and Rawles, 1940, p. 195), as well as of the specificity of the melanophores responsible for the contrasting color of the feathers of the two tracts (Ris, 1941, p. 60).

The technique was also used to check the earlier finding (Lillie and Wang, 1941) regarding the seat of determination of symmetry of regenerating feathers. Rotation of denuded dermal papillae prior to implantation into empty follicles made it possible to analyze the contribution of the separate components in the determination of feather orientation. Finally, an attempt was made to find the extent of the feather-forming capacity of the follicular epidermis by implanting denuded dermal papillae into empty and normal follicles at different levels of the follicular wall.

The purpose of this investigation, therefore, was (1) to delimit the morphogenetic functions of the epidermal and dermal components of the definitive papilla by (a) ascertaining the seat of tract specificity and (b) reinvestigating the seat of determination of symmetry in the regenerating feather and (2) to determine the extent to which general follicular epidermis, remaining after the removal of a papilla, is capable of feather regeneration.

II. MATERIALS AND METHODS

The experiments were performed on Brown Leghorn capons (except in three cases, see p. 341). Male chicks obtained from the Illinois Hatchery were castrated at the age of 4 weeks. The young capons were raised together and were kept on a well-balanced diet. Operations began when they were 4 months old and were

continued until they were a little over 1 year of age.

The following description of technique refers to the basic experiment; details pertaining to other types of operation will be given later. In searching for an agent for the destruction of the epidermal component of the papilla, alcohol, hot Ringer's solution, and weak solutions of alkali and inorganic acids were found to be unsatisfactory. Finally, salicylic acid dissolved in Collodion Flexible (Merck) proved successful. The ether and alcohol contained in this collodion evaporated rapidly on exposure to air, so that the viscous fluid promptly formed a film, i.e., a collodion coat, which could be readily stripped off. Immersion of isolated papillae for from 1 to 3 minutes in 25 per cent salicylic acid gave consistent results with a safe margin. The final formula of the mixture was fixed at 25 gm. of salicylic acid and 5 gm. of camphor (added as a germicide), dissolved in 100 cc. of the collodion. The standard duration of immersion was 1 minute, although a slightly longer exposure did not appreciably alter the results.

The most favorable sites for operation are the anterior breast and the extreme posterior saddle. (Hereafter breast and saddle will be designated as "B" and "S," respectively.) Feathers from these regions were plucked prior to each experiment, so that the regenerating feathers would be of the same regeneration age; usually, active B papillae were used after 14-35 days and S papillae after 14-45 days. For anesthesia, Nembutal (Pentobarbital Sodium, Abbott) was injected intraperitoneally (1 cc. per 5 pounds of body weight). All instruments were thoroughly cleaned, dried, and dipped in alcohol just before use. Solutions, cotton, and glassware were sterile.

For easier identification of operated

follicles horizontal rows of adjacent follicles, not more than ten in a row, were chosen. Operation proceeded from the most anterior one posteriorly. (See Lillie and Wang, 1941, Figs. 2, 3, 11, and 12, for a general orientation.) After sterilizing the skin with alcohol, the feather was plucked, and a slit about two-thirds of the depth of the follicle was made with scissors. The bottom of the follicle was lifted up with two pairs of pointed steel forceps under the binocular microscope; the papilla was brought into focus and then extirpated. During this process the papilla was held and lifted by a little collar of subdermal tissue left attached to it. Papillae of both B and S tracts, if removed in one operation, were placed in separate, labeled dishes containing Ringer's solution. From the latter the papillae were removed, one at a time, to a piece of filter paper to remove excess liquid, and then were immediately transferred to a glass slide, apical ends up. A drop of the salicylic acid mixture, not over 6 mm. in diameter, was then placed over the papilla. After 1 minute the papilla was removed from the film by its base and was returned to Ringer's solution. The excess follicular wall attached to the base of the papilla was trimmed so as to give a fresh base free from chemical contamination. Care was taken not to injure the basal portion of the dermal component of the papilla during trimming. It was then transferred once more to fresh Ringer's solution and left there for about 30 minutes. The treated papilla was now ready to be implanted into an empty follicle of the reciprocal tract, i.e., either B $\rightarrow$ S or S $\rightarrow$ B. Each denuded papilla was held lightly between the points of forceps while it was pushed toward the bottom of the recipient follicle, the incised wall of which was finally pulled together to heal. To insure successful incorporation of the

graft, the recipient follicle must not be left empty for more than 2 hours.

The arrangement of the operated follicles in rows removed the necessity of marking individual follicles. Adjacent feathers clipped immediately after the operation served as markers. As soon as

TABLE 1

TRACT SPECIFICITIES OF BREAST AND SADDLE FEATHERS IN BROWN LEGHORN CAPON

FEATURES		TRACTS	
		Breast	Saddle
Form	Shape	Rounded apex with broad vane	Acuminate apex with slender vane
	Average length	10 cm.	15 cm.
Structure	Vane	A uniformly interlocked vane formed by barbs carrying barbules along their entire length	An incoherent vane formed by barbs carrying barbules only in basal portions to form a "web," leaving margins of vane "lacy"
	Fluff	Structurally similar in both tracts	
		About one-half of total length	About one-third of total length
Pigmentation	Vane	Solidly black throughout	Golden brown except near the base, where a spot of black is usually present
	Fluff	Grayish in both tracts	
Mean growth rate....		2.3 mm. per day	1.8 mm per day

the follicles were healed, the skin about the first and last in each row was tattooed with India ink. The bird was examined at intervals; and after the feathers had completely regenerated, they were plucked and studied individually.

The characteristics which constitute the respective tract specificities of B and S are given in Table 1. All experimental feathers were classified on the basis of these characters only.

III. EXPERIMENTAL

A. EPIDERMAL ORIGIN OF TRACT SPECIFICITY

1. *The basic experiment.*—An average of from 3 to 4 weeks elapsed before feathers emerged from the operated follicles. From the very first their pigmentation was characteristic: feathers resulting from $S \rightarrow B$ were black, while those from $B \rightarrow S$ had a golden-brown color. This unmistakable acquisition of host tract pigmentation by feathers regenerating from denuded papillae was consistent throughout the experiments. Its significance, as contrasted with the invariable retention of donor tract pigment from untreated (intact) papilla-transplants between the two tracts, will be treated fully in the section on discussion. As the feathers grew longer, they revealed the typical structural characteristics of the host tract in spite of the fact that most of them were morphologically defective. When they were about 4 mm. long, those with defects limited to the apical portion showed a vane characteristic of the host tract. Those defective over their full length, i.e., tuft feathers, formed barbs identifiable as of host tract character despite the absence of a rachis. Later, when the fluff began to form, the general form and shape of the feathers were well established except for the tuft feathers. In each case these characters were found to be typical of the host tract: rounded tip and broad vane for those from $S \rightarrow B$, and an acuminate tip and narrow vane with a "lacy" margin for those resulting from $B \rightarrow S$. Microscopic study showed that in the tuft feathers grown on B the barbs possessed barbules throughout their length, whereas in those grown on S the apical portion was free from barbules, corresponding to the "lacy" margin of normal S feathers. This portion of

the tuft barbs, however, varied in length according to the total length of the barbs themselves. Thus, even in the absence of a rachis and a vane, one of the most characteristic differences of the B and S tracts was readily established. All other gross features—length, shape, fluff proportion, and pigmentation—conformed precisely to those of the host tract.

grown next to each of these was similarly measured. The results were conclusive: from the time of their emergence from the follicle all five feathers grew at exactly the same rate as their controls. Regardless of the origin of the dermal papilla, the growth rate was thus typical of the tract in which the feather was growing.

TABLE 2
RESULTS OF THE BASIC EXPERIMENT*

TYPE OF TRANSPLANTATION	EXPT. NO. (SER.)	NO. OF PAPILLAE TREATED	RESULTING FEATHERS					
			Approx. Normal	Missing Apex	Half-Vane	Apical Tuft	Tuft	Failed
S→B.....	34†	8	5	0	0	3	0	0
	38†	9	2	0	0	0	0	7
	47†	7	2	2	0	0	0	3
	125†	6	1	0	0	2	2	1
	107	6	2	1	1	1	0	1
	129	7	4	0	2	0	0	1
	131	7	0	0	0	2	1	4
	Total.....	50	16	3	3	8	3	17
B→S.....	Percentage.....	100	32.6	6.0	6.0	16.0	6.0	34.0
	34†	8	2	3	0	0	0	3
	38†	9	3	0	0	3	0	3
	47†	7	1	1	1	0	0	4
	125†	6	1	0	0	2	0	3
	33	6	0	0	1	1	1	3
	45	10	0	0	0	1	2	7
	127	8	0	0	2	1	3	2
Total.....	Percentage.....	54	7.0	4	4	8	6	25
	Percentage.....	100	13.0	7.5	7.5	15.0	11.0	46.0

* All resulting feathers listed in the table possess the specific characters of the host tract; no specific characters of the donor tract appear.

† Reciprocal transplantation.

In order to ascertain whether the growth rate of the composite feathers conforms to the dermal or epidermal component, three second-generation feathers growing on B (Expt. 129, No. 3; Expt. 107, No. 2; Expt. 47, No. 5) and two growing on S (Expt. 34, No. 2; Expt. 38, No. 1) were measured from the time their predecessors were plucked until they were completely regenerated (see Table 2). For comparison, one control feather

Normal B and S feathers take about 8–10 and 10–12 days, respectively, to emerge after plucking. But in the case of feathers regenerating from denuded papillae this lag was increased to as much as 4 weeks following implantation. The delay was, however, considerably lessened in the later generations, a fact which indicates a progressive disappearance of conditions unfavorable to regeneration introduced by the grafting process. For instance, of the five second-gen-

eration feathers used above for measurement of growth rate, none emerged within 17 days. Two B feathers emerged after 17 days, the other after 18 days; the two S feathers emerged after 20 days. The amount of delay that persisted in the second generation may be due to a prolonged subnormal physiological condition of the grafted papillae (cf. Lillie and Wang, 1943). These same feathers were examined again in their fourth generation. It was found that all three B feathers emerged only 2 days, the S feathers 6 days, after their controls.

The results of ten experiments (four reciprocal and six one-way transplantations) are summarized in Table 2. A total of sixty-two feathers was obtained from one hundred and four denuded papillae. These feathers were classified arbitrarily, according to the degree of defect, into five classes: (1) approximately normal, ranging from normal to apical defect not exceeding 1 cm.; (2) missing apex extending to any length of the vane; (3) distinctly half-vaned; (4) vaneless or with apical tuft; and (5) tuft (rhachisless) feathers (see Pls. I and II). It may be noted in passing that the apparent differences between the two tracts in regard to percentages of successful incorporations and of production of normal feathers are without statistical significance.

The data indicate that feathers from denuded papillae never manifest the characteristics of the tract of origin but invariably those of the tract in which they have grown. Nor was a mixture of donor and host features ever found. The tract specificity must therefore be attributed to the epidermal, i.e., host, component of the regenerating papilla. This is further borne out by the demonstration that the new epidermal coat of the graft is of host origin.

Direct evidence of the fact that the

epidermis of the treated papilla is destroyed and that the denuded graft subsequently acquires a new epidermal coat from the host follicle can be obtained by tracing the histological changes of the graft from the time of its implantation. Three series of transplants to B follicles, involving a total of twenty-one denuded S papillae, were made. Fourteen were recovered at 2, $4\frac{1}{2}$, 5, 6, and 7 days by dissecting out the entire follicles. These were fixed in Bouin's fluid, cut at $8\ \mu$ longitudinally and stained with Heidenhain's Iron Hematoxylin. Representative stages from the 2-, $4\frac{1}{2}$ -, and 7-day series are shown in Plate V, Figures 20, 21, and 22.

General observation, as well as histological study, of the recovered grafts revealed slight hemorrhage at the bottom of the wounded follicles, apparently dating back to the time of implantation. The resulting blood clot closed the cavity of the follicle distal to the apical end of the graft. No blood clot was found between the follicular wall and the graft, probably because of the pressure exerted by the follicular muscles. This pressure insures firm contact of the graft with all sides of the follicle except at its apex, a condition believed to favor successful incorporation.

Vascularization of the graft was established about 2 days after implantation. Sections recovered by that time gave evidence that the dermal papilla had been denuded and that epidermis of the host follicle was migrating over the implant. The basal portion of the graft was covered by epithelial cells, while its apical portion was still naked (see Pl. V, Fig. 20). That the direction of their movement was from base to apex was indicated by a "spearhead" of a single layer of epithelial cells in front of the much thicker sheet that had made a sharp turn from the bottom of the follicle up along

the surface of the graft. The activity of this invading epidermis appeared to have been stimulated by the presence of the graft, as indicated by the extensive thickening of the epidermal layer along the region of contact with the graft. Numerous mitotic figures were found around the entire cut edge of the follicle in direct contact with the graft in the region where the supplanting sheet of epidermis was thickest.

This epidermal replacement was advanced about half the length of the graft at $4\frac{1}{2}$ days (Pl. V, Fig. 21) and was completed by the end of the sixth or seventh day, when the entire graft became covered by new epidermis (Pl. V, Fig. 22). During this same period keratinization of the outer layers of the epidermal sheet occurred in the same manner as is observed in the outer margin of the collar in normal regenerating feathers. Meanwhile, the follicular cavity became filled with darkly stained keratin fibers. An initial differentiation of the epidermal cells into two layers appeared: inner cuboidal (Malpighian) cells with darkly stained nuclei and outer, more flattened cells destined to form keratin. The former layer normally gives rise to the differentiating zone of the future collar, although none of the series studied had as yet reached that stage, corresponding to the delay of emergence of feathers from grafts.

Up to 4 days after implantation the entire apical portion of the graft, in contrast to its basal portion, appeared bare and definitely necrotic. Farthest away from the source of vascularization and most exposed, the apical end obviously sustained the most severe damage. In a majority of cases it underwent degeneration and was eventually sloughed off.

A special effort was made to determine the fate of the destroyed epidermis. The sections revealed that a large part of the

dead epidermis had been stripped away from the graft, forming a part of the debris usually attached to its apex. Occasionally some remnants were seen to be loosely connected with the periphery of the graft, appearing as poorly stained, structureless fragments, often sandwiched in between the base of the graft and the incoming epidermis of the host follicle. Other cells of the injured epidermal coat had undoubtedly undergone necrosis; the apical end of the graft, especially in younger stages ($2-4\frac{1}{2}$ days) was crowded with cellular aggregates composed of erythrocytes and lymphatic elements. Not infrequently large macrophages were also found here. These myeloid elements were probably of host origin, since, in most cases where necrosis was extensive, host blood capillaries could be traced from the affected area to the base of the graft. It was largely by these two means that the dead epidermis was finally disposed of. A third remnant of the original epidermis was sometimes seen near the lower portion of the graft in the form of islands of faintly stained cells caught in the middle of the dermis at the boundary between the graft and the host subdermal tissues.

Careful examination of sections in every series gave no indication of any infiltration of host subdermal tissue into the graft. Since the graft, composed of lightly staining dermal tissue, can be readily distinguished from the more darkly staining host tissue, any invasion by the subdermis would have been readily detected. In all sections up to 7 days after implantation the boundary between the graft and the host follicle remained distinct, although they were completely fused. Hence, the histological evidence indicated that the newly established composite papilla consisted of the dermal component of the donor tract and the epidermal component of the host tract.

An important point that required examination concerned the possibility that the denuded graft might have stimulated the regeneration of a whole new papilla of host origin. Although the histological studies showed nothing of this kind, two experiments were devised to test the possibility more directly. In the first experiment two pulps from freshly plucked, actively regenerating B feathers were isolated (freed from barbs) and cut transversely into ten pieces of about 2 mm. each. Ten B follicles of the same bird were deprived of their papillae, and a piece of the isolated pulp tissue was implanted into each of them. After 5 weeks nothing could be detected externally; the follicles appeared as empty as "sterile" ones (i.e., those that normally remain inactive as a result of the complete removal of their papillae). Upon dissection it was found that each had healed without leaving any trace of the grafted pulp; the latter presumably had dried up. The experiment was repeated twice with consistently negative results.

In the second experiment subdermal tissue, freshly dissected out of B follicles, was cut into ten pieces of the size of papillae, and each of these was autoplastically implanted into a freshly prepared, empty B follicle. The operated follicles produced no feathers. When, at the end of 5 weeks, the follicles were dissected, no grafts were found; obviously, they had undergone the same fate as the pulp tissue. There was no sign of regeneration when the experiment was repeated.

The negative results of these two experiments strongly suggest that a follicle, once deprived of its papilla, cannot replace the latter. This is not surprising, in view of the fact that both the dermal core of the definitive papilla and its underlying subdermal tissue have reached irrevocable differentiation, providing no reserve from which the regeneration of a

new dermal papilla might draw. In this connection one recalls that structural defects in experimental feathers persisted throughout successive generations (Lillie and Wang, 1941, 1943), a condition apparently due to the inability of a papilla to repair its own losses. The fact that an empty follicle will remain inactive except in the presence of a grafted papilla, intact or denuded, demonstrates the existence of some specific affinity between the dermal core of a papilla and the follicular epidermis. The dermal component is thus indispensable for normal feather regeneration and is irreplaceable even though it is not specific between follicles of the same tract or between those of different (B and S) tracts.

In the light of the experimental evidence related above, one is led to the conclusion that all morphological distinctions (apart from symmetry) which, when taken together, constitute what we call "tract specificity" of a feather are determined exclusively by the epidermal component of the papilla, independent of the origin of the dermal component. The latter, being not tract specific, can consequently be interchanged between the tracts without affecting the tract specificity of the resulting feather.

The validity of this conclusion was further tested by transplanting an established composite papilla back to its original tract. In one experiment seven denuded S papillae were transplanted to B follicles and 3 days later implanted back to empty S follicles. Four of these failed; the remaining three gave rise to defective S feathers. The results indicated that the papillae involved in the double transfer had not been allowed sufficient time in the B tract to acquire a B epidermal coat before their return to the S tract. In a second experiment an equal number of S papillae were returned to S after having resided in B follicles for 8 days. These,

in sharp contrast to the results of the former group, yielded two definite, though defective, B feathers after a delay of 4 weeks, while the rest failed. This demonstrated the survival of the new epidermal component of B origin and its capacity to regenerate true to its tract regardless of the site of growth. In both experiments the unusually high percentage of failure possibly reflected the increased trauma involved in transplanting a treated papilla twice within a relatively short period.

2. *Other experiments.*—In order to further substantiate the significance of the basic experiment, several other experiments using slightly modified techniques were carried out. Some of them which involved local treatment of papillae—i.e., the *in situ* and “hot-needle” experiments—required the previous establishment of normal B and S papillae in their reciprocal tracts. Forty-six normal B feathers on S and fifty-two S feathers on B (a total yield of 74 per cent) were thus obtained by transplanting intact papillae, with some of the attached follicular wall and subdermal tissue, from one tract to the other (Lillie and Wang, 1941). In one operation, however, the bases of the transplants were trimmed under the dissecting microscope so as to rid them of all attached tissue. Eight such trimmed B and S papillae, each consisting solely of the dermal core and its epidermal covering, were then reciprocally transplanted. They yielded six normal B feathers on S and five S feathers on B. These differed in no way from those produced by untrimmed papillae in regard to structure, pigmentation, and capacity for subsequent regenerations. This indicates that all criteria of tract specificity are determined by the regeneration cells which constitute the epidermal component of a papilla. These cells evidently represent

an inexhaustible source of successive generations of feathers.

When fully regenerated, these first-generation feathers from reciprocally transplanted B and S papillae, displaying their original donor tract specificities, were plucked. When the second generations had reached a length of some 40 mm., each duplicating its predecessor, they were again plucked. Papillae of second-generation feathers were used as a precaution in order that the first generation might insure the full consolidation of the tract specificity of the particular papilla. The active papillae remaining after the second plucking were then treated as follows: The follicle was slit open nearly to the bottom; a large drop of the denuding reagent (three to four times the volume used in the basic experiment) was spread over the papilla *in situ*, including the area around its base. After 1, 3, 5, or 7 minutes the resulting collodion film was removed without washing the affected area. The flaps of the follicular wall were closed as usual. As a rule, the treated papillae were slow in regenerating; the amount of delay was approximately proportional to the duration of the treatment. In a few cases as much as 6 weeks passed before the first sign of regeneration, while the most rapid cases took about 3 weeks.

The results of this series of experiments are given in Table 3. They seem to lack uniformity, apparently because of a new factor involved, the duration of exposure. After short treatment feathers of the same character emerged as in the preceding generations, while longer treatment increased the tendency toward reversal of the former tract character. Obviously, treatment of 1 minute failed to kill the epidermis of the papilla; hence the original donor tract specificity was preserved. Three minutes still permitted the recovery of the donor epidermis, al-

though in two cases successful partial killing of the epidermis with subsequent replacement from the host tract resulted in chimaera feathers which displayed both B and S features. Five minutes brought about complete destruction of the epidermal cells, together with serious damage to the dermal core, leading to defective feathers characteristic of the host tract as a result of the replacement of epidermis from the host tract. Seven

in the basic experiment, successful destruction of the epidermal component and subsequent replacement of local host epidermis were responsible for the observed alteration of tract specificity.

The two chimaera feathers in the last experiment (see Table 3) were obtained accidentally. Such cases can be produced deliberately by partial destruction of the epidermal component of a papilla either prior to its transplantation to the recip-

TABLE 3
RESULTS OF THE *in situ* EXPERIMENT

TYPE OF ORIGINAL FEATHER	EXPT. NO. (SER.)	NO. OF PAPILLAE TREATED	DURATION OF EXPOSURE (MIN.)	RESULTING FEATHERS			
				Breast	Saddle	Chimaerae	Failed
S on B.....	46	7	1	○	{ 4 normal 3 defective }	○	○
	48	5	3	○	4 defective	1	○
	41	4	5	1 tuft	1 defective	○	2
	43	4	7	1 tuft	○	○	3
B on S.....	30	5	1	{ 4 normal 1 defective }	○	○	○
	31	6	3	{ 2 normal 3 defective }	○	1	○
	37	6	5	○	4 tuft	○	2
	35	4	7	○	1 tuft	○	3

minutes' exposure killed the entire papilla except in two cases, in which tuft feathers with host tract specificity were obtained.

The difference between the results of this experiment and those of the basic experiment seems to be primarily due to the different treatment. The papillae treated *in situ* had an advantage in that the vascularization was undisturbed. But an exposure of from 5 to 7 minutes killed not only the superficial cells but a substantial portion of the dermal core as well. This accounts for the structural defects of those feathers in which the treatment was sufficient to change the tract specificity. In the *in situ* experiment, as

rocal tract or after it has been incorporated there. The latter was accomplished by the "hot-needle" experiment. Actively regenerating second-generation feathers, similar to those used in the *in situ* experiment, were plucked, and their follicles were opened as usual. While the papilla was held with forceps below its base, a heated steel needle was quickly applied to one side of the exposed papilla, chosen at random. Twelve B papillae incorporated in S and seventeen S papillae incorporated in B were so treated. The results of the experiments are given in Table 4.

The percentage of failures from the heat-treated papillae was high: eleven

out of twenty-nine cases (38 per cent). Eight chimaerae (five on B and three on S) and ten nonchimaeric feathers were produced. Three of the chimaerae grown on B had the general shape of an S feather, but toward the base of the vane black-pigmented barbs predominated on one side. One interesting feature of all the chimaerae grown on B is the 180° spiral twisting of their axes. The twist may be ascribed to an immediate effect of the intense heat, since it entirely disappeared in the second generation. One of the three chimaerae grown on S was a better

by the epidermal coat did not involve the replacement by the epidermis of the host follicle, whereas manifestation of the host tract specificity was the result of a complete destruction of the epidermis followed by host replacement. In both cases the structural defects of the feather were presumably due to heat damage to the dermal core of the papilla.

The other method of production of chimaera feather was by partially immersing an isolated papilla in the denuding reagent, thereby achieving unilateral destruction of the epidermal component.

TABLE 4
RESULTS OF THE "HOT-NEEDLE" EXPERIMENT

TYPE OF ORIGINAL FEATHER	EXPT. NO. (SER.)	NO. OF PAPILLAE TREATED	RESULTING FEATHERS			
			Breast	Saddle	Chimaerae	Failed
S on B.	51	10	2 defective	1 tuft	3	4
	53	7	1 defective	{ 1 tuft 1 defective }	2	2
B on S.	54	6	1 defective	2 tuft	1	2
	57	6	1 defective	0	2	3

specimen in that the B and S components were more nearly equal. The B barbs appeared in one side of the vane, while the rest of the feather resembled an S feather (Pl. III, Fig. 12). The other four chimaerae (two on B and two on S) are principally B feathers with an S margin in the vane, evidently the result of random killing of the epidermis. Significantly, no chimaera of B margin on S vane was obtained (cf. Lillie and Wang, 1943).
The ten nonchimaeric feathers were all defective. The fact that some of them were B and others S regardless of the tract in which the treatment took place is significant. It indicates that the amount of epidermis killed was purely a matter of accident. Donor tract specificity was retained if the injury sustained

The host follicle would be expected to replace only that fraction of the epidermis which had been destroyed, with the resulting production of a composite coat composed of both B and S cells. An isolated B or S papilla, after being rolled over a piece of filter paper, was placed, with its axis horizontal, in a drop of the denuding reagent on a small watch glass. The size of the drop was so adjusted that one lateral half of the papilla would protrude from the fluid and thus remain free from injury. The papilla was removed after 1 minute, washed in Ringer's solution, trimmed at the base, and transplanted in the usual manner to an empty follicle of the reciprocal tract. No effort was made to determine whether the immersed half was a dorsal, ventral,

or lateral one. The results are given in Table 5.

The percentage of total failures was reduced to 24 per cent (seven out of twenty-nine cases), compared to 46 and 34 per cent, respectively, for the $B \rightarrow S$ and $S \rightarrow B$ grafts of totally immersed papillae in the basic experiments. This was probably due to the fact that none of the partially immersed papillae was killed by the treatment itself. The resulting feathers on either tract were of two distinct types: chimaerae and defective nonchimaeric feathers, the latter being invariably characteristic of the donor tract, in

TABLE 5
RESULTS OF THE PARTIAL-IMMER-
SION EXPERIMENT

TYPE OF TRANSPLANTATION	EXPT. NO. (SER.)	NO. OF PAPILLAE TREATED	RESULTING FEATHERS			
			Defective Breast	Defective Saddle	Chimaerae	Failed
$S \rightarrow B$	49	7	0	5	1	1
	50	7	0	4	0	3
$B \rightarrow S$	52	7	3	0	2	2
	55	7	4	0	2	1

contrast with the defective feathers characteristic of either the donor or host tract obtained in the "hot-needle" experiment. That there was no complete acquisition of host tract specificity is ascribable to the retention by the partially immersed papilla of half of its epidermal coat. The replacement of killed epidermis may take place either from host follicular epidermis or from cells of the intact papilla epidermis, these conditions giving rise to chimaerae and nonchimaeric feathers of donor tract specificity, respectively. The relative frequency of incidence of about 3 to 1 in favor of the latter indicates that there was a greater chance for the killed epidermis to be replaced by the surviving epidermal coat of the transplant than by the host follicle. This was later confirmed by an experiment in which dorsal B and S

half-papillae were transplanted to empty follicles of reciprocal tracts. While all six such S transplants in B follicles gave rise to pure S feathers, two chimaerae were obtained on S from a total of twelve B grafts. There was, thus, only 13 per cent (two out of eighteen cases) in which the cut surface of the transplants became subsequently covered by the host follicular epidermis; in the remaining cases (87 per cent) the replacement took place from the cells of the graft.

All the chimaerae (see Table 5) that resulted from partially immersed grafts were free from twisting. This shows that the latter phenomenon is characteristic only of the heat treatment. The chimaera grown on B is slightly longer than a normal B feather; its vane, the apex of which is missing, is about two-thirds of the total length. On the left side of the vane the golden-brown pigment predominates except for a few black barbs near the base. On the right, B features predominate save for a distinct S margin. Such a chimaeric pattern is clearly accidental, unparalleled by any of the chimaerae produced by combinations of B and S half-papillae (Lillie and Wang, 1941, 1943). All the four chimaerae grown on S carry S margins in their vanes, two unilaterally and one bilaterally (Pl. III, Figs. 11, 13). They differ in total length, fluff proportion, and general form; but all are more or less intermediate between B and S.

All the chimaera feathers thus far produced, irrespective of the method used, bear additional evidence that tract specificity is determined by the epidermal component alone. They also demonstrate what the basic experiment failed to bring out, namely, that B and S regeneration cells, once they have been exposed to the influence of the dermal papilla, are capable of autonomous realization of their own tract specificity even when inter-

spersed with fragments of the other and regardless of the proportion of the two kinds of cells present in the mosaic epidermal component.

An experiment using picric acid as a denuding reagent was done before the salicylic acid mixture was established. Isolated papillae were totally immersed in saturated picric acid for 1 minute, thoroughly washed in Ringer's solution, and transplanted into follicles of the reciprocal tract. One capon served for the exchange of eight B and S papillae. The results from the eight S → B transplants were one good chimaera, which has been reproduced for five successive generations; five defective B feathers; and two cases of failure. The eight B → S grafts gave three chimaerae and two defective S feathers, while the rest failed. It is significant that the tract specificity of the defective feathers derived from both tracts were invariably those of the host tract. This indicated that the picric acid had completely killed the epidermal component in seven out of eleven cases, while the chimaerae were the result of replacement of partially destroyed epidermis of the graft. The latter resembled those derived from the partial-immersion treatment in that they were free from any twisting of the axis, but they were rather unique in the variation of structure in successive generations (see below). These results further substantiate the established fact that, whenever a composite epidermal coat is formed over a dermal core of a papilla, the resulting feather will be a chimaera displaying both B and S characteristics in proportion to the amount of the two epidermal cells which go into the making of the mosaic pattern.

3. *Successive generations of feathers.*—Special attention was given to the structure of the successive generations of the feathers resulting from each of the above

experiments. Some of them are now in their sixth or seventh generation. The majority showed a persistent structural pattern with only insignificant variations in the type of defect—if any. In a limited number of cases, however, there was a general tendency toward (1) a progressive reduction in intensity of the defect and (2) a slight increase in the length of the feather (cf. chimaera feathers in Lillie and Wang, 1943). A few typical examples are given below: Experiment 107, No. 6, derived from a denuded S papilla transplanted to B, began as a typical half-vane B feather and remained so in the second generation, with only slightly increased barbs; but in its third generation it changed to a longer and almost perfect B feather, with just a few apical barbs missing (Pl. IV, Figs. 14, 15, 16). No. 5 of the same experiment was half-vaned in the first generation only; it became a relatively normal B feather in the second generation and has remained so. Four of the chimaerae from the picric acid experiment showed progressive changes in structure. Of the one grown on B, the first generation had severe apical defects; the right side of the vane was mostly S, while its left side carried only a spot of S margin. Its second and third generations were both about twice as long as the first generation, and with a much better developed S component in structure as well as in pigmentation (Pl. IV, Figs. 17, 18). The three chimaerae grown on S showed considerable increase in length in later generations, although no appreciable difference in structure. A similar tendency toward increase in the extent of the S component and total length of the feather was also manifested by the two chimaerae derived from partially immersed papillae (Expt. 55). These changes in structure, invariably in the direction of decreasing defectiveness, are believed to be due to some internal

readjustment within the injured dermal component of the papilla.

In addition to changes in structure and length, we have already noted (p. 335) the complete disappearance of axial twisting in second-generation chimaerae derived from the application of heat to papillae *in situ*. Another phenomenon commonly observed in tuft feathers was their increase in both length and number of barbs, although a shift from the tuft type to a less defective category (e.g., half-vaned) never occurred in later generations. This suggests that, although repair of damage is possible, apparently it has a limit.

These differences occurring in later generations have not hitherto been noted in feathers which resulted either from portions of intact papillae or from recombined papillae (Lillie and Wang, 1941, 1943) and hence must be regarded as significant. Apparently, there exists a difference between defective papillae involving loss of parts and those injured by means of heat or chemical effect. The results of the *in situ* experiment (p. 333) have given us some inkling as to the possible nature of this difference, although the real mechanism leading to the repair of the latter papillae is not clearly understood.

B. ROLE OF THE DERMAL COMPONENT

The technique of transplantation of denuded papillae was utilized to ascertain the seat of determination of symmetry in regenerating feathers. The results of the basic experiment were not adequate for this purpose, since all the denuded papillae used were oriented at random. Specially designed experiments were accordingly carried out on four capons. On each bird, twelve S papillae, treated with the denuding reagent by total immersion, were transplanted to empty B follicles with their axis rotated

180°, i.e., with the dorsoventral axis reversed. Six comparably treated B papillae, transplanted to their own follicles with similarly reversed axes, served as controls. Before the papillae were detached from their follicles, they were marked by a tiny notch cut at the dorsal apical end.³

Results of this series of experiments are presented in Table 6. The forty-eight S papillae yielded thirty B feathers on breast (including four tuft feathers which had no bearing on the question of orientation), while eighteen approximately normal B feathers were obtained from the twenty-four B papillae (62.5 and 75 per cent yield, respectively). The feathers were classified according to the position of the rhachis. Class 1 includes those feathers with the rhachis normally oriented in the median dorsal position or rotated by less than 45° in either direction. Class 2 represents those with the rhachis in the left quadrant; Class 3, those in the right quadrant. Class 4, with the rhachis in the ventral quadrant, comprises the feathers with completely reversed orientation.

The most striking result is that none of the feathers of which the denuded papillae had been rotated by 180° had a normal or near-normal orientation. Over 50 per cent showed the reversed orientation that corresponds to the inversion of the grafted papillae. The results are thus comparable to those obtained by Lillie and Wang (1941, p. 111), who used a totally different technique. They proved that the feather formed by the replacing host tract epidermis owes its orientation and symmetry to an orienting influence of the dermal core. What remains to be

³ An attempt was made to cut the notch at the ventral side of the apex instead of the dorsal side, in order to avoid damaging the dorsal field; but it was unsuccessful because of the difficulties arising from the obscured view.

explained is the high incidence of moderately oriented feathers in Classes 2 and 3. It seems probable that these feathers arose as a consequence of deviations from the intended reversed orientation, which might have been due either to inaccurate marking or to a secondary shifting in the position of the grafts resulting from the bird's movements. At any event, these results confirm the conclusion of Lillie and Wang (1941) that the field of symmetry is located exclusively in the der-

papilla was transplanted into an empty follicle—the epidermis of the host follicle can supply an equivalent functional substitute. Thus, the results of the basic experiment alone provide sufficient indication that feather-forming capacity is at least not limited to the regeneration cells. The problem then becomes: To what extent does the follicular epidermis as a whole possess feather-forming capacity? Is any part of it capable of feather regeneration in the presence of the indis-

TABLE 6
RESULTS OF TRANSPLANTATION OF DENUDED PAPILLAE, 180° ROTATED

ORIENTATION OF THE RESULTING FEATHERS	STRUCTURE						
	From Denuded S Papillae					From Denuded B Papillae	
	Approx. Normal	Missing Apex	Half- Vane	Apical Tuft	Per Cent	All Approx. Normal	Per Cent
Class 1 (dorsal quadrant).....	0	0	0	0	0	0	0
Class 2 (left quadrant).....	2	3	2	0	27	5	28
Class 3 (right quadrant).....	4	0	1	0	19	3	16
Class 4 (ventral quadrant)....	5	7	0	1	50	10	56
Undetermined.....	0	0	0	1	4	0	0
Total.....	11	10	3	2	100	18	100

mal component of the papilla. With this finding, the morphogenetic roles of the two components of the papilla are thus clearly defined.

C. FEATHER-FORMING CAPACITY OF
FOLLICULAR EPIDERMIS

Lillie and Wang (1941, pp. 109, 130) regarded the regeneration cells (p. 325, n. 2) as totipotent so far as the feather is concerned. This view is further substantiated by the experiment described on page 333. It was shown above (p. 332) that the regeneration cells, when removed, could not be replaced by the remaining follicular epidermis in the absence of a dermal papilla. But, when the latter is present—e.g., when a denuded

pensable dermal papilla, or is this capacity limited? The following experiments were designed to answer these questions.

In the first experiment denuded active S papillae were transplanted to different levels of the follicular wall of empty B follicles. A small hole, about the diameter of a papilla, was made on the ventral or dorsal wall of the recipient follicle without removing any of the incised tissue. The graft was placed well into the hole with its base securely anchored (see text Fig. 1). A total of sixteen denuded S papillae were transplanted each into a separate follicle with the center of the graft located at approximately the 1-, 2-, 3-, or 4-mm. level, measured from the

bottom of the follicle. From the sixteen grafts (four at each of the four levels) two feathers were obtained at the 1-mm. level, one each at the 2-mm. and 3-mm. levels, but none from the 4-mm. level.

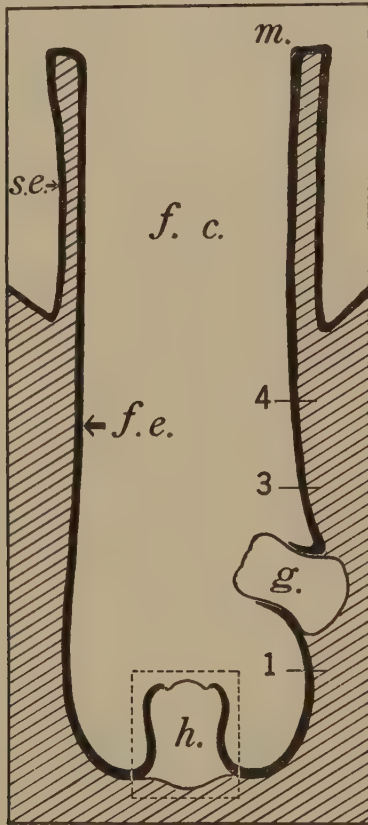


Fig. 1.—Diagram of a longitudinal section of a breast follicle showing a denuded saddle papilla (g.) grafted to its wall 2 mm. from the bottom. The host papilla (h.) may be left intact or removed; the latter condition is indicated by the dotted lines. Subdermis is represented by oblique parallel lines. Key: f.c. = follicular cavity; f.e. = follicular epidermis; s.e. = skin epidermis; m. = mouth of the follicle; 1, 3, and 4 indicate the 1-mm., 3-mm., and 4-mm. levels at which similar grafts were implanted.

All four feathers were typical B feathers, similar to those obtained from the basic experiment. As controls, four untreated S papillae were similarly transplanted to each of these four levels; and, in addition, another four to the remaining apical portion of the follicular wall. Comparable

results were obtained but with better yield: three S feathers at the 1-mm. level and two at each of the next three levels, while none was produced at the most apical portion. Since normal feathers were obtained from denuded papilla-grafts implanted anywhere up to the 3-mm. level, it would seem that the follicular epidermis as high up as that level has a feather-forming capacity equal to that of the regular regeneration cells.

Production of feathers at these same levels of the follicle was next attempted by the implantation of denuded papillae into follicles, the papillae of which were retained intact (text Fig. 1). This experiment was believed to be crucial, since, if successful, two wholly independent feathers would grow in the same follicle. Altogether, nineteen operations were made on a total of four birds, with either the dorsal or ventral follicular wall used as a site of implantation. The results of this series of experiments are listed in Table 7.

In each of the three successful cases two entirely independent feathers were obtained. The one derived from the graft showed certain defects, and its orientation bore no relation to that of the feather derived from the intact host papilla. For instance, in the one obtained on the White Leghorn hen (Table 7) the two feathers faced each other with their dorsal surfaces, a condition which never occurs in twinning resulting from operation (Lillie and Wang, 1941). In the two cases on the Brown Leghorn capon, on the other hand, the feathers were oriented with the ventral side of one facing the dorsal surface of the other (Pl. IV, Fig. 19). Since the two partners are attached at their calami, the distance between the bases of the calami (inferior umbilici) should express the position of the graft relative to the normal papilla. This was actually so for the host and donor papillae in the case on the White Leghorn hen.

They were found, upon dissection, to be exactly 2 mm. apart from each other. These results prove that the follicular epidermis in question can, under the stimulus of the dermal graft, form a feather not only in the absence of the host papilla but even while the latter is engaged in normal regeneration.

Finally, experiments were devised to test whether or not extrafollicular epidermis has feather-regenerating capacity when provided with a dermal papilla. The region directly above the breast is ideal for this purpose because of its rich vascularization and the protection of-

with its edge securely fastened to the skin by a Scotch tape. The bird was caged alone, and an effort was made to see that the watch glass remained in place for at least 4 days.

Two Brown Leghorn capons were used in one experiment. One of them carried twelve denuded active B papillae, and the other, nine denuded active S papillae, transplanted into skin openings above the breast. The result was entirely negative: none of the transplants yielded any feather. Within 1 week the skin wound closed and the grafts appeared to have dried up.

TABLE 7
RESULTS OF GRAFTING DENUDED PAPILLAE TO THE FOLLICULAR
WALL OF NORMAL FOLLICLES*

Level of Grafts	Bird Used	No. of Cases	Donor Tract	Host Tract	Position of Grafts	Successful Cases
1 mm.....	Brown Leghorn capon	{ 2	S	B	dorsal	1
		{ 2	B	B	ventral	1
2 mm.....	White Leghorn hen	5	B	B	dorsal	1
3 mm.....	Brown Leghorn cock	5	B	B	dorsal	0
4 mm.....	Brown Leghorn cock	5	B	B	ventral	0

* A case was considered successful when two feathers developed from one follicle.

fered by the wing. A row of small cuts about 2.5 mm. long and 4 mm. apart were made on the skin along the course of a sizable blood vessel. Just prior to transplantation the skin openings were slightly enlarged along their edges, and all the subdermal tissue directly underneath was removed. The opening was then moistened with Ringer's solution, and a denuded papilla was transplanted into it with its base firmly placed against some exposed capillaries; the whole graft was finally covered up with the incised skin fold. For 2 hours the grafts were continually moistened with Ringer's solution, while the bird remained under anesthesia. After that, in order to prevent the grafts from drying, a watch glass was inverted over the operated area

For a control another capon was similarly operated on, with the transplantation of six intact active B papillae and two active S papillae, both types of papillae being untreated. Five days after the operation both of the S grafts and four of the six B papillae were found to have commenced regeneration. These grafts appeared to have resumed regeneration very quickly, apparently as a result of rapid re-establishment of vascularization. At the end of a month three of the four B feathers had formed normal follicles, while the fourth one was not growing equally well. Of the two S feathers, one was healthy, with a completely developed follicle, whereas the other had shown signs of regression. The net outcome was, therefore, one S and

three B feathers, which are now in their sixth generation.

The contrast shown by these two experiments in regard to the capacities of denuded and intact papillae to produce feathers may be indicative of an inability of the extrafollicular skin epidermis to respond to the dermal component of the papilla, although mechanical or other local conditions arising from the absence of the protective epidermal lining, as well as of the follicle, may have caused the denuded grafts to disintegrate.

The potentiality of the apical half of the follicular epidermis has been left undetermined, owing to difficulties involved in the operation. Whatever it may be, the results of these experiments seem to favor the conclusion that the feather-forming capacity is retained by the definitive follicular epidermis only.

IV. DISCUSSION

Feather regeneration as an induction system.—The feather is a product of active collaboration between the epidermal and dermal constituents of the papilla. The two components are mutually indispensable, since neither can produce a feather alone. Specifically, then, what does each of them do? Obviously, the epidermis furnishes the building material, while the dermal papilla sets this material in operation as well as directs its organization. The action of the dermal papilla has been described as having the attributes of a "field" (Lillie and Wang, 1941). The fact that no feather can be formed in the absence of a dermal papilla shows that the latter imparts a specific activation to the epidermal cells, without which these cells would only remain "idle." The field next induces a "secondary" field in the developing epidermis in correspondence with its own polarity by initiating the formation of a

series of parallel barbs dorsally, whereby the position of the future rachis is determined (Lillie and Wang, 1941, p. 106). Concurrently with the establishment of the symmetry of the feather, or prior to it, the epidermis becomes a self-differentiating system. Defective papillae lacking a complete field (Lillie and Wang, 1941) or those injured by heat and chemicals lead invariably to structural defects either as a result of deficient field action or indirectly due to upset of the developmental mechanism, such as, for instance, the derangement of the tangential movement of barbs. It is thus clear that, although the feather is differentiated from the follicular epidermis, its symmetry and orientation are, nevertheless, under the control of the dermal field. Aside from these morphogenetic properties, the dermal papilla also performs important physical and physiological functions (Lillie, 1940) in providing a scaffolding for the support of the feather cylinder and the continual production of pulp throughout the period of regeneration as a means of distributing nutrition to the growing cells of the feather.

The dermal papilla, however, apparently does not impart any new characters to the epidermal cells but merely transforms them into whatever the latter are already intrinsically prepared to be. We have seen, for instance, that the denuded breast and saddle papillae, i.e., pure dermal components, do not differ in their organizing properties, since they can be interchanged to collaborate with the epidermis of the other tract without affecting the tract specificity of the regenerate derived from such a composite papilla. This means that, whereas the influence of the dermal component is of a general nature and acts presumably on both kinds of follicular epidermis in the same manner, the response from the latter is, in contrast, very specific. For the breast

epidermis produces only breast feathers, regardless of whether it is provided with the dermal papilla of its own or of another tract; and, similarly, saddle epidermis produces only saddle feathers. In general, then, feather regeneration is in line with many known developmental systems in early embryogenesis (Weiss, 1939, pp. 321-437), in which a mesodermal tissue incorporates and effects the differentiation of an overlying ectoderm. While, in such a dual system, the ultimate character of the organ in question is determined by the responding ectoderm in accordance with its potency, the polarity, symmetry, and the normal developmental mechanism of the differentiating organ come under the influence and control of the mesoderm. Tissues endowed with the latter properties, to which the dermal papilla evidently belongs, are often referred to as "inductors." Experiments now under way in this laboratory which are directed more specifically to localizing and delineating the papilla-inductor (see Lillie and Wang, 1944) may soon yield more definite information regarding its nature.⁴

The question arises as to wherein lie the ultimate differences between the epidermis of the breast and the saddle, respectively. The cells themselves do not appear to differ morphologically. Yet they must differ physiologically, since, faced with the same dermal stimulus, one type develops into breast, and the other into saddle, feathers, which, as we know, differ specifically in their growth rate, shape, pigmentation, and the structure of barbs. These differences, which constitute the so-called "tract specificities," must be ascribed to the specific properties that each epidermal region acquired during its embryonic differentiation. One

recalls the work of Weiss and Amprino (1940), who demonstrate that perfectly homogeneous and morphologically indistinguishable prescleral mesenchyme of 4-day chick embryos, when cultured *in vitro*, produces cartilage of the specific scleral variety. The potencies of any tissue or cells, therefore, cannot and should not be judged by their appearance but may be learned only from the outcome of their performance.

Possible relationship between the differential growth rate and pigment production of feather tracts.—We do not know whether the persisting differences in the growth rate of breast and saddle feathers can be traced back to correspondingly different growth potentials of their respective follicular epidermis or whether the difference represents something acquired during the course of differentiation. Much significance has been attached to it in the interpretation of hormone threshold (Lillie and Juhn, 1932; Juhn, Faulkner, and Gustavson, 1931; Juhn and Gustavson, 1930) and in the determination of the rhythmic pattern in the Barred Plymouth Rock feathers (Montalenti, 1934; Willier, 1941). A possible correlation between basal metabolism and growth rate of feathers is demonstrated by Lillie and Wang (1940). Recently, the latter authors (1943) showed that in a certain composite collar from combination of breast and saddle half-papillae the observed replacement of saddle cells by those of the breast might be due to some intrinsic property, possibly again related to their higher growth rate.

Pigment cells have been demonstrated to be of neural-crest origin in both amphibians and birds⁵ (Du Shane, 1938, 1939; Dorris, 1939; Eastlick, 1939a and 1940; Danforth, 1937, 1939; Hamilton, 1940, 1941; Rawles, 1939 and 1940a;

⁴ See Lillie (1942) for a review of the recent work on feather development and for the complete citation of its literature.

⁵ Rawles (1940b) demonstrates the neural-crest origin of melanophores in the mouse also.

Ris, 1941; Watterson, 1941, 1942; Willier and Rawles, 1940). Attention is now centered on the specificity of melanophores and the physiology of production of pigment. The unsettled issue in the dimorphic Brown Leghorn fowl is whether in the male (or castrates of both sexes) there are two types of melanophores for the breast and saddle, respectively, or whether only one type is involved in the production of both the black and the brown pigments, possibly under different conditions of the substratum (epidermis).

The indication from the present results seems to favor the latter theory. We have seen that feathers derived from untreated papillae retain the pigmentation of their original tract irrespective of the site of growth, whereas those from denuded papillae exchanged between breast and saddle invariably take on the pigmentation of the host tract. What does this contrast imply? It suggests that, whatever their source, the melanophores appear to be dependent in some specific manner upon the feather-forming epidermis. The feathers derived from the untreated grafts retain the pigmentation of their tract of origin because the entire papillae survive, while those derived from the denuded papillae become pigmented in accordance with the host tract because their new epidermal component is of the latter origin. Clearly, the epidermis is the controlling factor: without exception, the pigmentation follows the tract which contributes the epidermal component of the papilla, whether in normal regeneration or following grafting. In short, the pigment is determined concurrently with the other morphological criteria of tract specificity.

The situation is analogous to the "dependent" melanophores of the white axolotl (Du Shane, 1935, 1939). In this unpigmented axolotl the melanophores are present but will not produce melanin

in the absence of a specific substance; the latter, though normally lacking in the animal itself, can be supplied by epidermis taken from a pigmented species such as *Amblystoma punctatum*. Earlier, Harrison (1935) found a similar situation in the limb of *Triturus torosus* embryos. As to the nature of this substance present in the epidermis necessary for the production of specific pigment by the potential melanophores, Du Shane (1935) suggests a specific oxidase (dopa oxidase) comparable to that found in the mammalian epidermis originally investigated by Bloch (1917).⁶

In case of the follicular epidermis of the fowl under consideration, a certain physiological provision necessary for the oxidation of dopa into black melanin appears to be lacking in the saddle follicles. This might be due to the production of a substance by the saddle epidermis, which acts as an "inhibitor" to the synthesis of black melanin (cf. Dorris, 1939), or it might be that the chain reaction effecting the oxidation of dopa into melanin comes to a halt at an intermediate stage at which the oxidation product is a brown pigment, such as is characteristic of the saddle feather (cf. Dorris, 1942, p. 117). The latter alternative may be the result of a mere quantitative difference—for instance, in the oxygen content of the oxidases. In any event, we assume that the undifferentiated melanoblasts of the two tracts are physiologically the same and hence produce the same propigment, dopa; the determination as to which of the two (black or brown) melanins the latter will form upon oxidation lies with the intrinsically different properties of the follicular epidermis of the two tracts. The fact that the breast and saddle differ in the growth rate of their feathers leads one to surmise

⁶ For recent discussion on this subject see Du Shane's review (1943).

that the ultimate cause for the differentiation of pigments might have arisen from a difference in the metabolic rate of the cells. This hypothesis presupposes, of course, the existence of a certain threshold, and the rates above and below it are responsible, respectively, for the black and brown pigments. If the growth rates of breast and saddle feathers (Table 1) represent the metabolic rates of their respective follicular epidermis, a direct correlation may be drawn to attribute the different pigments to their respective growth rates. But, as yet, no such demonstration is available.

A point of important bearing on pigmentation concerns the source of melanophores in the feather germ. Willier and Rawles (1941, p. 195) originally suggested that probably the regeneration cells represent the source of reserve melanophores. Although our results do not indicate which of the two components of the papilla may be their permanent residence, they do render it doubtful that the mesoderm could be a source of chromatophores in feather regeneration. The question will be decided by the experiments of Foulks, whose preliminary report (Foulks, 1942) revealed that "melanoblasts arise outside of the feather germ and penetrate the collar after migrating through the dermal papilla." This seems to imply that neither the dermal nor the epidermal component permanently harbors the pigment cells but rather that the melanoblasts migrate anew in each regeneration from the subdermis into the collar by way of the dermal core. Significantly enough, this finding helps one to explain how trimmed papillae reciprocally transplanted between breast and saddle tracts could furnish the donor-tract pigment without exhaustion (p. 333). It is probable that a new supply of local (host) melanoblasts are utilized in later generations which

are of the same physiological type as those of the donor tract. Such an interpretation favors the "monophyletic" theory.

The above considerations conform with the works of Hamilton (1940, 1941), who stressed the autonomy of black and red melanophores in the red breeds of fowl studied *in vitro*. Our results agree with his statement that "a precursor cell might be incorporated in the feather germ and then diverge either in the black or red direction *in situ* due to physiological differences or external conditions in its environment" (Hamilton, 1941, p. 295; Dorris, 1942, p. 117); but, in addition, our results point definitely to the follicular epidermis as constituting perhaps the primary factor implicated in the differentiation of the two types of melanophores (cf. Willier, 1943, pp. 141, 142). A slight divergence of opinion concerns the mode of action of hormones: While the cultivation of relatively free melanoblasts *in vitro* perfectly justified Hamilton (1941, p. 296) in concluding "that hormones are not acting secondarily through some intermediate cell type" when definitive follicular tissue is not present, this statement may not necessarily apply to hormones when the latter are allowed to come into direct contact with the follicular cells *in situ*. This concept receives indorsement from Willier (1943, p. 141) in his interpretation of Hamilton's work. The induction of female growth rate in the capon (Juhn, Faulkner, and Gustavson, 1931, p. 78) simultaneously with plumage feminization caused by the injection of female sex hormone speaks favorably for the possibility that hormones may exert their specific effect on melanin synthesis through the medium of follicular epidermis.

Reinterpretation of related work having a bearing on the epidermal origin of tract

specificity.—Eastlick (1939b), in his study of feathers obtained from limb transplants made between embryos of different breeds of fowl, concluded that invading host mesoderm was probably responsible for the mixed host and donor types of the feathers as well as for their growth rate. In the light of the evidence presented above, this could hardly be the case; instead, it must have been due to the actual incorporation of host follicular epidermis in the transplants. This interpretation is in full accord with the results of Willier and Rawles (1940). In all their cases the transplanted skin and pure mesoderm alike supplied the specific donor melanoblasts to the graft region, while the follicles, including entire papillae were of host origin. Consequently, the resulting feathers invariably have and retain the form, structure, and growth rate of the host, while their pigment is produced by donor pigment cells until the time of their replacement by host melanophores.

The prolonged maintenance of donor color pattern in the mosaic feathers resulting from skin grafts (Danforth and Foster, 1929; Danforth, 1937, 1939) has not been fully accounted for, although Willier and Rawles (1940, p. 125) already correctly pointed to the different techniques used by them and by Danforth as the fundamental cause. More specifically, in Danforth's experiments the skin grafts carried over not only abundant melanoblasts but with them also complete follicles. During the operation certain papillae along the path of the scissors were probably incised; and, by chance, these split papillae of host and donor origin might have subsequently fused and healed together, thereby giving rise to composite papillae.⁷ Another

alternative was the formation of composite papillae by incised papillae of the graft and host follicular epidermis, or vice versa, along the line of healing. This is more probable than the actual fusion of part-papillae of both the host and the graft, in view of the improbability of bringing together, by chance, in one follicle cut papillae of both origins. One recalls (p. 336) that chimaera feathers of such a derivation were actually experimentally produced. In any event, only by either of these two modes of origin could we explain the fact that the mosaic feathers occurred only at the margins of the graft, never in the graft itself or in the purely host area. There is no doubt that such mosaic feathers incorporated the genetically specific host and donor types of melanoblasts as has been proved histologically to be the case (Danforth, 1937, p. 463). But, if the migration of the pigmentoblasts alone was responsible for the mosaic character of the color pattern in these feathers, a replacement of the donor melanophores by those of the host was not only certain to occur but should take place sooner than in the feathers obtained by Willier and Rawles (1940). The persistence of the mosaic pattern in Danforth's feathers must, therefore, be explained on a material basis, namely, that the host and donor melanophores are each accompanied by its own epidermal complement of the same origin. The mosaic feathers are thus comparable to the chimaerae produced by combination of parts of papillae (Lillie and Wang, 1941, 1943), in that the composition of their papillae remains stable, once it is established. Unfortunately, however, no reciprocal grafting of skin was made between tracts of contrasting morphology, such as, for instance, between the breast of the Barred Plymouth Rock and the saddle of the male Leghorn. The latter ex-

⁷ This was the original explanation of Danforth and Foster (1929, p. 465), but it was later abandoned by Danforth (1939, p. 173).

periment would be a crucial test for the ventured interpretation of the origin of the mosaic feathers.

Differences in the nature of defective papillae and their consequences.—Finally, the observed structural changes in later generations of the experimental feathers, which, so far, constitute the first exceptions to the usual maintenance of structural patterns in feathers derived from defective papillae (Lillie and Wang, 1941) and those from combined half-papillae (Lillie and Wang, 1943), call for an explanation. To understand this contrast, one may turn to the *in situ* experiment (p. 333). There we have noted the importance of the duration of exposure: a duration which would have been sufficient to kill the epidermis of isolated papillae proved to be without effect when applied to papillae treated *in situ*. This indicated that the chemical effect from 1 minute's exposure was perhaps not instantaneously lethal to the tissue, particularly the dermal core, but that its destruction would ensue when subsequently followed by a period of lack of vascularization, as happened to many of the denuded grafts in the basic experiment. The papillae treated *in situ*, however, retained their normal vascularization and thus required several times the normal duration of exposure in order to be killed. A shorter duration resulted in complete or partial recovery of the affected papilla. Thus, it appears possible that cells subjected to heat or chemicals may remain quiescent and functionless, i.e., dormant, for a certain length of time; and, since the dermal core sustains relatively less injury than the superficial epidermal coat in any treatment, the chance for recovery in the dermal core is greater. This is quite in line with our observation that most of the changes in later generations concerned length of the feather and kind of structural defects—which, as we know,

are expressions of the relative intactness of the field in the dermal papilla—and, further, that the changes are always in the direction of diminishing defectiveness. The increase in length or improvement of structure never extend beyond the third generation, a fact which lends support to the idea of a gradual but limited recovery from damage sustained during the treatment.

Needless to say, the ability to repair temporary injury is limited to only those papillae which do not suffer material losses from actual removal. Portions of a papilla remaining after an operation, owing to lack of power to replace the missing part, will remain as defective as they were at the beginning; and hence the structural pattern of the feather it produces remains invariable too.

Other possible causes for the structural variations are mechanical in nature. A physical hindrance to the developmental process of the feather, such as might be caused by the presence of a scar on the surface of a healed graft or by prolonged adherence of debris to the incorporated graft, may so derange the regular mechanism of feather development as to produce defects in the first generation which otherwise would not be formed. Such being the case, the better-developed later generations would naturally be the result of subsequent removal of the physical obstruction.

V. SUMMARY

1. Morphologically contrasting breast and saddle tracts of Brown Leghorn capons were used to separate the relative roles of the epidermal and dermal components of the papilla in the determination of tract specificity of regenerating feathers.

2. Isolated papillae were immersed in a solution consisting of 25 gm. of salicylic acid and 5 gm. of camphor dissolved in

100 cc. of Collodion Flexible (Merck) for 1 minute, to destroy their epidermal coat.

3. When breast or saddle papillae, completely denuded of their epidermal component, were autoplastically transplanted to empty follicles of the reciprocal tract, they invariably produced feathers characteristic of the host tract in growth rate, shape, form, structure, and pigmentation.

4. Histological study of the grafts revealed that the process of replacement of dead graft epidermis by epidermis of the host follicle was completed on about the sixth day following implantation. The possibility of substitution of the whole grafted papilla by host material was ruled out by (a) histological evidence and (b) implantation of small pieces of pulp and isolated follicular subdermal tissue into empty follicles, neither of which provoked formation of a new papilla. The composite character of the experimental papilla, consisting of the dermal core of the donor tract and a newly acquired epidermal coat from the host tract, has thus been definitely established. The change in tract specificity of the denuded papillae must be ascribed to their having become recoated from the reciprocal tract.

5. Essentially the same results were obtained by denuding papillae *in situ* after they had been well established following reciprocal transplantation. Full host tract specificity was acquired only in cases in which the original epidermal component had been completely de-

stroyed. Similarly transplanted papillae, when partially denuded by heat injury or partially immersed in the denuding reagent, produced chimaerae with mixed breast and saddle characters, in addition to various types of defects.

6. It is concluded, therefore, that tract specificity is a function of the epidermal component of the papilla alone. The dermal components of breast and saddle are not tract specific.

7. It is thus revealed that epidermal cells of different regions, in spite of the absence of morphological and histological distinctions, differ in their morphogenetic properties and, hence, give rise to different products in response to the same stimulus.

8. Denuded papillae, transplanted in random dorsoventral orientation, give rise to feathers of corresponding orientation. When rotated 180° at the time of implantation, most of the resulting feathers were inverted. This demonstrates that the factors determining the symmetry of the feather reside in the dermal component.

9. The capacity to form a feather was found to be present at least in the entire basal half of the follicular epidermis. Extra-follicular (skin) epidermis, however, could not be demonstrated to possess this capacity.

10. The role of the dermal component and the bearing of the present results on a possible relationship between the follicular epidermis and pigment production are discussed.

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PLATE I

FIG. 1.—Normal breast feather of Brown Leghorn capon. Dorsal view. $\times \frac{3}{4}$.

FIGS. 2-5.—B feathers from denuded S papillae transplanted to B follicles, representative

of the results of the basic experiment. From left to right: approximately normal (Expt. 34, No. 4), missing apex (Expt. 47, No. 2), half-vane (Expt. 129, No. 1), and tuft, or rhachisless (Expt. 125, No. 2). Dorsal view. $\times \frac{3}{4}$.

PLATE II

FIG. 6.—Normal saddle feather of Brown Leghorn capon. Dorsal view. $\times \frac{3}{4}$.

FIGS 7-10.—S feathers from denuded B papillae transplanted to S follicles, representative

of the results of the basic experiment. From left to right: approximately normal (Expt. 38, No. 5), defective apex (Expt. 34, No. 1), half-vane (Expt. 127, No. 4), and apical tuft (Expt. 38, No. 2). Dorsal view. $\times \frac{3}{4}$.

PLATE III

FIG. 11.—Chimaera feather from a partially denuded B papilla transplanted to S follicle (Expt. 55, no. 3). It resembles an S feather in length and fluff proportion and carries a "lacy" (S) margin on both sides of the vane; but the entire apex (black) and the greater central portion of the vane are B. Dorsal view. Natural size.

FIG. 12.—Chimaera feather from a heat-treated B papilla after its establishment in an

S follicle (Expt. 57, No. 4). It retains the length and fluff proportion of a B feather, while its complete right vane-half and a narrow margin on the left are S. Dorsal view. Natural size.

FIG. 13.—Chimaera feather from a partially denuded B papilla transplanted to S follicle (Expt. 52, No. 3). Except for a large S portion in its left vane-half and its increased total length, the rest of the feather is B. Dorsal view. Natural size.

PLATE IV

FIGS. 14, 15, AND 16.—First-, second-, and third-generation feathers, respectively, from a completely denuded S papilla transplanted to B follicle (Expt. 107, No. 6). These feathers, completely B, show continual decrease in the intensity of structural defects (see p. 337). Dorsal view. $\times \frac{4}{5}$.

FIGS. 17 AND 18.—First- and second-generation chimaerae from an S papilla treated with picric acid and transplanted to B follicle (see p. 337). The S characters are represented by

only a few S barbs in the right vane-half in the first generation, but they make up the entire right vane-half, as well as a margin on the left, in the second generation. Dorsal view. $\times \frac{4}{5}$.

FIG. 19.—Two feathers (see p. 340) grown in the same B follicle. The one at the left is from the host papilla; that at the right from a denuded S papilla transplanted to the dorsal wall of the follicle 2 mm. up from its bottom. Their orientation, one on the top of the other, is accidental. Dorsal view. $\times \frac{4}{5}$.

PLATE V

FIG. 20.—Longitudinal section of a denuded S papilla in a B follicle, 2 days after implantation. Approximately the basal third of the follicle is shown (Figs. 20-22). Epidermis of the host follicle has covered the graft, on its right, to the level indicated by the arrow, while on its left no replacement has begun, although the supplanting epidermal sheet has already made the upward turn (*arrow*). On this side the graft is bounded by a darkly stained streak which consists mostly of remnants of dead graft epidermis. Necrosis has not set in; the apex of the graft is fused with the blood clot in the follicular cavity formed at the time of implantation. Heidenhain's Iron-Hematoxylin stain; 8μ . $\times 95$.

FIG. 21.—Longitudinal section of a denuded S papilla in a B follicle, $4\frac{1}{2}$ days after implantation. Replacement of host epidermis has advanced much further (*arrows*). The graft is necrotic particularly at the apex, which is crowded with islands of dead epidermis intermingled with host lymphocytes and other

myeloid tissues (note a large cluster at the right). Delafield Hematoxylin and eosin stains: 8μ . $\times 95$.

FIG. 22.—Longitudinal section of a denuded S papilla, 7 days after implantation to a B follicle. The graft, having survived the necrosis, is now completely covered with epidermis. Nevertheless, its base remains clearly demarcated from the host subdermis. The entire graft (dermal) appears much healthier than in the earlier stages. The small vacuole-like structures at its very apical end immediately beneath the newly formed epidermal lining represent spaces formerly occupied by the remnants of graft epidermis; one such island is shown (*arrow*). The darkly stained structures filling the follicular cavity right above the incorporated graft consist chiefly of debris, including remnants of the blood clot and keratin fibers formed by the outer layers of the follicular epidermis. Heidenhain's Iron-Hematoxylin stain; 8μ . $\times 95$.

PLATE I



PLATE II



PLATE III



PLATE IV



